Associations among food and protein intake, serine dehydratase, and plasma amino acids

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Relationships among liver serine-threonine dehydratase activity, plasma amino acid concentrations, and food and protein intake were studied in growing rats undergoing adaptation to high-protein diets. Serine-threonine dehydratase activity was low initially but increased as protein intake rose above the amount required for rapid growth. Plasma amino acid concentrations were greatly elevated 1 day after animals were fed high-protein diets but, with the exceptions of leucine, isoleucine, and valine, decreased thereafter as serine-threonine dehydratase activity increased. Food intake of rats fed high-protein diets was depressed initially when serine-threonine dehydratase activity was low and plasma amino acid concentrations were high. Food intake rose subsequently as enzyme activity increased and plasma amino acid concentrations decreased. Ingestion of a high-protein diet apparently results in a series of homeostatic responses: initially plasma amino acid concentrations rise and food intake falls; despite this, protein intake is elevated and activities of enzymes of amino acid catabolism increase; subsequently plasma amino acid concentrations decrease and food intake returns to normal.

Adaptation of the rat to a high protein intake involves complex physiological and biochemical alterations including depressions in food intake and weight gain, hypertrophy of the liver and kidney, enhanced gluconeogenesis, and elevated activities of many tissue enzymes (1, 2, 9, 12, 13, 15, 16, 24). Prominent among the metabolic adaptations are elevations in the activities of enzymes of amino acid catabolism such as arginase (2, 14, 16, 25), other urea-cycle enzymes (25), ornithine transaminase (20, 29), glutamate-oxalacetate transaminase (17, 19, 25), glutamate-pyruvate transaminase (17, 22, 23, 25, 28), tryptophan pyrrolase (11, 19), tyrosine α-ketoglutarate transaminase (23), glutamic dehydrogenase (32) and serine-threonine dehydratase (3-6, 19, 20). The increase in the ability of rats adapted to a high-protein intake to degrade amino acids (31) and the high correlation between urea excretion and the activities of urea-cycle enzymes (2, 25-27) suggest that these enzymatic adaptations contribute importantly to the maintenance of homoeostasis in animals fed a high-protein diet.

Ashida and Harper (2) suggested that the depression in food intake observed when rats are fed a high-protein diet may be the initial homeostatic response to a protein intake that exceeds the capacity of the animal to catabolize amino acids. Although depressed food intake of rats fed a diet imbalanced with respect to one indispensable amino acid has been shown to be associated with changes in the pattern of plasma amino acids (10), no attempt has been made to examine relationships among plasma amino acid concentrations, food intake, and the activities of enzymes of amino acid catabolism in the rat during the period in which food intake is depressed as the result of elevated protein intake.

In the present investigation the relationship between changes in plasma amino acid concentrations and serine-threonine dehydratase activity was examined at intervals in rats undergoing adaptation to a high-protein intake. Serine-threonine dehydratase was selected as being representative of enzymes that increase in activity under conditions in which amino acid catabolism is enhanced (3-7, 19, 20). The hypothesis underlying the investigation was that, for some time after the protein content of its diet had been greatly elevated, the rat would be unable to oxidize rapidly the large excess of amino acids it ingested with the result that plasma amino acids would increase and food intake decrease; then as adaptation to a high-protein intake progressed the enzymatic capacity of the rat to oxidize the excess of amino acids would increase, plasma amino acid concentrations would decrease and normal food intake would be re-established. Evidence for such an association among protein intake, plasma amino acid concentra-
tions, the activity of an enzyme of amino acid catabolism, and food intake is presented in this paper.

EXPERIMENTAL PROCEDURE

Animal and diets. Male rats of the Holtzman strain weighing 90-100 g were individually housed in suspended wire-bottom cages in a room equipped with an automatic 12-hr light control. The rats were offered the diets, prepared as agar gels (21), and water ad lib. The diets contained in percent by weight: vitamin-test casein (General Biochemicals), 5, 25, 50, or 75%; corn oil, 5%; mineral mixture (21), 5%; vitamin mixture (21), 0.5%; choline chloride, 0.2%; cornstarch and glucose monohydrate in a ratio of 1:1 to make up 100%. The 5% casein diet contained an additional 0.3% L-methionine and 0.2% of L-threonine.

Experimental plan. All rats were fed the 5% casein diet for 10 days. They were then divided into four groups of equal average weight (134-135 g) and were fed the 5, 25, 50, or 75% casein diet for 1, 2, 3, 6, or 14 days. Daily dry matter intake and weight were recorded for each animal. After the diets had been consumed for the designated number of days, four or five animals from each group were lightly anesthetized with ether during the first 1.5 hr after the beginning of the daylight period; the abdominal cavity was opened and blood was withdrawn by inserting the needle of a heparin-rinsed syringe into the heart through the intact diaphragm. Using this procedure the rats were kept alive throughout the blood sampling. The liver was then immediately excised, blotted on filter paper, weighed, and chilled in 0.14 M KCl. The stomachs of most animals contained some food at the time of sacrifice.

Amino acid assay. Plasma amino acid concentrations were determined using the Technicon amino acid analyzer. Equal quantities of plasma from each rat within a group were pooled and subsequently deproteinized with an amount of 15% sulfosalicylic acid to make the concentration in the resulting supernate 3% in sulfosalicylic acid. The plasma supernates contained considerable quantities of asparagine and glutamine which could not be adequately separated from the first four amino acid peaks on the chromatogram. Therefore, each sample was heated for 3 hr at 100 °C in a sealed ampule with an equal volume of 3% sulfosalicylic acid. This procedure hydrolyzed the amines and resulted in reasonably good separation of aspartic acid, threonine, serine, and glutamic acid.

Enzyme assay. Serine dehydratase activity was determined by a modification of the method of Friedland and Avery (5) in which the rate of disappearance of DPNH was followed at 340 mμ and 30°C. A portion of each rat liver was homogenized in 0.14 M KCl and centrifuged for 45 min at 27,000 X g and 4°C. The reaction mixture contained 2.53 X 10^-4 M L-serine, 4.23 X 10^-4 M DPNH, and 5.37 X 10^-4 M pyridoxal 5’ phosphate.

RESULTS

There was a sharp but transitory reduction in both food consumption (Fig. 1) and weight gain (Table 1) during the first few days after rats were switched from the low- to the high-protein diets. On day 1 the food intakes of the rats fed 25, 50, and 75% casein diets fell to 58, 33, and 28% respectively, of the amount consumed by the rats fed the 5% casein diet (Fig. 1). The weight gains of animals fed the 50 and 75% casein diets were also reduced on day 1 (Table 1). Thereafter, the food consumption of all groups fed the high-casein diets increased rapidly until day 6 and continued to increase until the end of the experiment for rats fed the 50 and 75% casein diets. The cumulative weight gains of rats fed the 75% casein diet were significantly less than those of the rats fed 25 or 50% casein on days 2, 3, (P < .01), and 6, (P < .05), but not on day 14 (Table 1). After day 6 the food intakes and the growth rates of the groups fed the 25, 50 and 75% casein diets did not differ significantly.

The protein consumption of each group is shown in Fig. 2. The protein intake of rats fed 5% casein remained constant for 14 days, while that of groups fed 25, 50 and 75% casein increased markedly on day 1, despite the substantial decreases in food intake (Fig. 1), and continued to increase rapidly for 3–6 days.

Alterations in liver serine-threonine dehydratase activity are shown in Fig. 3. Enzyme activity was related to the protein content of the diet; activity remained
unchanged throughout the study in the livers of rats fed the 5% casein diet but increased rapidly during the first 3–6 days, then more slowly, in the livers of rats fed the higher casein diets. On the 1st day, enzyme activity in the livers of the rats fed the 75% casein diet increased significantly (P < .01) above that of rats fed the 5, 25, or 50% casein diets. By day 14 the enzyme activity in the livers of rats fed the 25, 50, or 75% casein diets had increased 25-, 135-, and 255-fold, respectively, above that for rats fed the 5% casein diet. The period of low enzyme activity on day 1 for groups fed the high-protein diets coincided with the time of appetite (Fig. 1) and growth depression (Table 1).

Figure 4 shows that the relationship between serine-threonine dehydratase activity and total protein consumed was not linear. Enzyme activity did not increase appreciably until protein intake was above approximately 3 g/day.

The concentrations of threonine, serine, leucine, and total amino acids in the plasma of rats fed the four levels of protein for 2 weeks are shown in Fig. 5. The concentration of the total plasma amino acids of the rats fed the 5% casein diet increased significantly (P < .01) above that of rats fed the 5, 25, or 50% casein diets. By day 14 the enzyme activity in the livers of rats fed the 25, 50, or 75% casein diets had increased 25-, 135-, and 255-fold, respectively, above that for rats fed the 5% casein diet. The period of low enzyme activity on day 1 for groups fed the high-protein diets coincided with the time of appetite (Fig. 1) and growth depression (Table 1). Table 1. Effect on growth of feeding 5, 25, 50, or 75% casein to rats previously fed 5% casein for 10 days

<table>
<thead>
<tr>
<th>Percent of Casein in Diet</th>
<th>Mean Cumulative Weight Changes, g/rat</th>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>5%</td>
<td>5.6*</td>
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<tr>
<td>25%</td>
<td>4.9</td>
</tr>
<tr>
<td>50%</td>
<td>1.3‡</td>
</tr>
<tr>
<td>75%</td>
<td>-0.9†‡</td>
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* Each value represents the mean of the remaining rats (22-25 on day 1 to 4-5 on day 14). † Significantly different (P < .05) from rats fed 5% casein. ‡ Significantly different (P < .05) from rats fed 25% casein.

The plasma leucine concentration (Fig. 5) is representative of the behavior of the branched-chain amino acids. In rats fed the 5% casein diet the concentrations of these amino acids remained unchanged throughout the experiment (Fig. 5 A), but in rats fed the 50 and 75% casein diets the concentrations rose as protein intake increased (Fig. 5, C, D). The plasma concentration of leucine on day 14 for rats fed the 75% casein diet was eight times the zero-day value while valine and isoleucine increased nine- and sevenfold, respectively. The higher value for total amino acids on day 14 for rats fed this diet compared with those for rats fed 25 and 50% casein is due to the higher concentrations of the branched-chain amino acids; when the values for leucine, isoleucine, and valine were subtracted the resulting concentration of total amino acids was near the zero-day value (Fig. 5 D).

Since serine dehydratase and threonine dehydratase appear to be a single protein in the rat (5, 6, 18) the plasma concentrations of both substrates, serine, and threonine, are of interest. In general, the changes in the concentrations of serine and threonine followed the pattern for the total amino acids (Fig. 5). Serine and threonine concentrations rose in rats fed 5% casein during the 14-day period. The inclusion of 0.2% of L-threonine in the 5% casein diet in an attempt to make it more adequate and the low enzyme activity of the rats fed this diet (Fig. 3) may account for the gradual increase in plasma threonine concentration. The threonine content of this diet would nevertheless be equivalent only to that of a 10% casein diet. When the rats were switched to the high-casein diets plasma serine and threonine concentrations increased sharply on day 1. The smaller increase on day 1 in the plasma threonine concentration of rats fed the 75% casein diet (18 µmoles vs. 74 and 87 µmoles/100 ml of plasma for rats fed 50 and 25% casein diets, respectively) may be a reflection of the elevation that had occurred in serine-threonine dehydratase activity in the livers of the 75% casein group by this time (Fig. 3). Plasma serine concentration did not show this type of behavior on day 1, possibly because the rat can synthesize serine. Following the initial elevation, serine concentration returned to the
FIG. 3. Liver serine dehydratase (referred to as serine-threonine dehydratase in the text) activity of rats previously fed 5% casein for 10 days and then fed 5, 25, 50, or 75% casein. Serine dehydratase activity (µmoles DPNH/min per 100 g body wt) is plotted vs. days on the diet. Each point represents the average enzyme activity of 4 or 5 rats. Zero-day value is 0.4 ± 0.1 units. Values for groups fed the 5% casein diet ranged from 0.35 to 0.47 units throughout the 14-day period. ●, 5% casein; □, 25% casein; ○, 50% casein; □, 75% casein. X, mean ±SE; SE values for rats fed 5% casein were negligible.

FIG. 4. Relationship between serine dehydratase activity and protein intake. Serine dehydratase activity (µmoles DPNH/min per 100 g body wt) is plotted vs. protein (g) consumed per day. Each point represents the average enzyme activity and the average protein intake of the 4 or 5 rats on which the enzyme determinations were done. Numbers on the figure indicate the day of the experiment represented by the points. Other values grouped in the lower left-hand corner are for days 1, 2, and 3. ●, 5% casein; □, 25% casein; ○, 50% casein; □, 75% casein.

The zero day value in 3 or 6 days in the plasma of the rats fed 50 and 75% casein diets and in 14 days in those fed the 25% casein diet. Threonine concentrations fell below the zero-day value in these time periods. The intakes of serine and threonine on day 14 (Fig. 5, D–D) were 2.5- to 13.3-fold higher than the average intakes of these amino acids on day 0.

Thus plasma amino acid concentrations were elevated when amino acid degrading activity, as indicated by serine-threonine dehydratase activity (Fig. 3), was low and declined as the activity of this enzyme increased.

**DISCUSSION**

The physiologic and metabolic responses of the rat to an increase in the protein content of its diet appear to be related to its capacity to degrade amino acids. The present results suggest that the sequence of the events is as follows: the activities of enzymes of amino acid catabolism in the rat adjusted to a low-protein intake are low (17, 19, 22, 23, 25); when the protein content of the diet is increased protein intake increases, the amounts of amino acids absorbed are greater than those to which the animal is adjusted and plasma amino acid concentrations rise greatly; within a short time food intake is depressed, but despite this, the amount of protein consumed exceeds that consumed when the protein content of the diet is low. Within 24–48 hr serine-threonine dehydratase, taken here as an indicator of the behavior of many of the enzymes of amino acid catabolism, begins to increase, plasma amino acid concentrations begin to fall and food intake to rise. Within a few days enzyme activity rises to a maximum that is roughly proportional to protein intake; food intake increases and plasma amino acid concentrations diminish to values close to those obtaining at the time of initiation of the high-protein feeding even though protein intake remains greatly elevated. Thus alterations both in food intake and in amino acid degrading capacity contribute to the maintenance of homeostasis during the shift from a low to a high steady-state rate of amino acid metabolism. During the course of the transition, growth rate increases appreciably so the greater deposition of body protein would also contribute to the maintenance of homeostasis especially in animals receiving intermediate levels of protein.

The observation that the activity of serine-threonine dehydratase is influenced by the protein content of the diet is in agreement with that of Pitot et al. (19), but the relationship between enzyme activity and protein consumption is not uniform. The response resembles that observed for tyrosine-α-ketoglutarate transaminase (23) in that enzyme activity remained low until the amount of protein consumed exceeded some critical quantity (Fig. 4). This presumably represents the point at which the quantity of amino acids consumed exceeds the amount that can be readily utilized for protein synthesis. When enzyme activity was low on day 1 an intake of 3 g or more of protein, the amount of crude...
Food intake depression was associated with low serine-threonine dehydratase activity and high concentrations of plasma amino acids, while food intake stimulation was associated with increasing enzyme activity and decreasing plasma amino acid concentrations. These results support the suggestion (2, 13) that the amount of protein an animal will consume voluntarily depends upon its ability to metabolize amino acids within a short period of time.

The branched-chain amino acids in the plasma of rats fed the 50 and 75% casein diets continued to increase with time while all other amino acids decreased. One can only speculate as to the reason for this. Apparently, the capacity of the animals fed the higher casein diets to degrade these amino acids was exceeded since elevated concentrations were not observed in the plasma of rats fed the 25% casein diet. These observations suggest that, in animals fed a high-protein diet, the degrading enzymes for branched-chain amino acids respond more slowly or to a lesser extent than the degrading enzymes for other amino acids, or that the branched chain amino acids are transported to the tissues more slowly.

REFERENCES


HOMEOSTATIC RESPONSES TO DIETARY PROTEIN


